CONSERVATION OF INVERTEBRATE BIODIVERSITY:
THE ROLE OF EX SITU PRESERVATION OF GENETIC MATERIAL

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Although conservation of biological diversity in situ is of primary importance, ex situ conservation is also important. Despite increased awareness of the need for habitat preservation, extinction of some species appears inevitable. If a species becomes extinct, a fragment of biodiversity is destroyed. However if its DNA is suitably preserved, all is not lost — DNA can be used to study the molecular make-up of an organism, and as a source of genes of scientific or practical value. DNA contains the information which underlies the idiosyncratic features of species and individuals — genes specify the structure of diverse molecules and control cell growth and development. From DNA sequences we can follow the tracks of evolution. This wealth of information from wild species is one of our most important resources. The introduction of gene banks to house source material and to provide databases is therefore a logical step. These will serve two functions: (a) a resource for exploring biological diversity and evolutionary history; and (b) a resource of increasing importance for the development of biotechnology. Gene banks can make DNA readily available to researchers throughout the world, to facilitate the development of molecular tools for further studies of biodiversity and to aid conservation research. ☐ *Gene banks, molecular biology, conservation, biodiversity, genotype, DNA storage, biotechnology.*

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Despite the increased awareness of the need for habitat preservation, the main issue of biodiversity conservation is not so much whether there will be significant loss of species, but rather the extent to which this can be prevented. While habitat preservation may be the immediate goal of those concerned with the preservation of biological diversity; present financial and human resources will limit this to high priority areas. In other areas, there seems little doubt that some extinction is inevitable. Particularly in the case of invertebrates, where so many microhabitats harbour such an enormous range of diverse forms, there will be significant loss of species and whole communities, some of which may disappear before they are examined in detail.

Each fragment of 'biodiversity' is the result of millions of years of evolution — a resource that is priceless to science, and of increasing economical importance as products and genes from wild species are utilised for the benefit of mankind. All of this information need not be lost when a species becomes extinct; part is contained in the genome and can be preserved if the integrity of its DNA is maintained. DNA can be thought of as an incredibly complex blueprint describing the molecular make-up of an entire living organism. DNA contains a wealth of information — intri-

cate details of the structure of molecules, control of gene expression (determining embryonic development and tissue differentiation) and even the evolutionary history of any individual.

It could be said that 'biological diversity is encoded in DNA'. DNA contains all the information which underlies the idiosyncratic features of species and individuals — genes specify the structure of diverse molecules and control cell growth and development. Because of the enormous, recent advances in molecular genetics, it is now possible to decode this information and increasingly to integrate it. At a time when the biological diversity of the planet is both actually and potentially under threat, we need to gather this information and preserve it as an information base for future generations. Even if species are lost, the information in the DNA will ultimately itself be of great intrinsic value as people start to take a molecular genetic perspective on biology, evolution, diversity and function. As we explore the diversity of these systems and identify genes that relate to interesting developmental pathways, genes that catalyse unusual biochemistries or which encode resistances or adaptive responses for particular environments, gene banks will have significant intrinsic value as scientific resources and then ultimately as biotechnological

resources. It is the information encoded in DNA that we need to keep for its own sake as a resource for science, as a resource for biotechnology, and if necessary for posterity in case the living species becomes extinct.

DNA — THE SOFTWARE OF LIFE

In the past, because of the origins of the science, genetics has been viewed with either a biochemical perspective, or a population perspective. These intellectual trends which tend to be looking at either the biochemistry of DNA or inheritance in populations, do not consider DNA in the way that it really functions — as biological software. The analogies of all life forms with a computer are very strong. In any cell or organism, the cellular machinery, which produces RNA and protein from DNA, is analogous to hardware, being similar throughout the biosphere. DNA is then the software, the component that is put into the computer to obtain a particular output — the specific molecules, cell types and developmental processes that result in different individuals and species. There is then a different version of the software for each individual. Each version (the DNA sequence) can be considered to have an element of data (coding sequence) and an element of programming (sequences that control gene expression).

With the advent of molecular genetic techniques, this information can be read from DNA, our molecular software. These techniques use components of the cellular hardware as tools to read DNA sequences and obtain information on the structure of proteins and control of gene expression. In site directed mutagenesis and transgenic analysis the software is modified and put back into the cellular hardware, to gain information on the function of molecules, tissue differentiation and embryonic development. We can obtain still more information by comparing the DNA software (both structural and programming elements) of different individuals and species. We can thus examine the molecular basis of disease resistance and productivity of agricultural breeds, as well as the molecular basis of biodiversity. Because slight alterations in the software are inherited, pedigree analysis can be carried out by DNA fingerprinting. As the software is modified step by step over many generations we have a record of evolution and can gain information on evolutionary paths and phylogenetic relationships. There is a wealth of information and huge amounts of data stored in this software of life,

which we have only just started to explore using the recent explosion of molecular tools to read and analyse the data.

Putting all this information together, the Earth's biological database is enormous. The total number of living species is at least 5×10^7. Excluding viruses, genome sizes range from 600,000 base pairs in the most primitive cells (mycoplasma) through to more than 10^{10} in some plants. As the latter contain a lot of repetitive DNA, an average coding content is likely to be about 10^8 base pairs of DNA. This means the Earth's genetic database is of the order of 5×10^{16} bits of information. In one sense this is an over-estimate as many genes are shared in common between organisms. We are looking at variations upon themes; but the idiosyncratic variations on themes is the basis of biodiversity. What else is biological diversity but a continuum of variation on a theme, with lineages?

The biological database is probably the most important information on the planet. We need to preserve and utilise this database which can be accessed using DNA and RNA resources.

EXPLORING BIOLOGICAL DIVERSITY THROUGH DNA SEQUENCE

The exploration of the genetic basis of life is being driven by the key models, particularly by the human genome project. Even though we do not realise it this is the first step in exploring biological diversity. It is necessary to first to understand how key models work, whether it be *E. coli*, *Bacillus subtilus*, *C. elegans*, *Saccharomyces cerevisiae*, *Drosophila*, mouse, man or *Arabidopsis*; so that the precise and detailed information we are going to gather in large amounts from all the diverse species of the planet can be integrated into some sort of structure. Around the world there are now genome projects that involve a whole range of species including insects. Once the genomes of key representatives of selected phyla within the biological spectrum have been defined there will be a very rapid lateral expansion of the knowledge base into related species. The question then becomes, 'What is different about each species?'; and we will be exploring biological diversity at its roots — at the genetic level. We need to preserve DNA for the future exploration of diversity. In that context in our part of the world there are many invertebrate species not yet identified, as well as many species that are highly endangered. It is not just a question

of scientific exploration, but getting to these species before they disappear.

GENE BANKS — PRESERVATION OF GENOTYPE

When dealing with preserved specimens, the phenotype (what we can see) is just a few percent of what is there. The unique biochemistry, mechanisms of cell differentiation and embryological development, phylogenetic position, and evolutionary history are all hidden, but written in the DNA. When a species becomes extinct, it is a tragedy if that genetic information is not preserved. Gene banks and ex situ collections of DNA are not going to replace phenotypic collections. In order to understand the genotype, molecular studies need to be integrated with extensive studies of phenotypes and habitats. In many parts of the world, there are well established institutions collecting invertebrate samples, and we have benefited from these locally. It is important that those organisations collect and preserve genes as well.

There has been some criticism of museums and herbaria in the past that they are just mausoleums, but if they are treated as genetic reservoirs, a more active face is provided for these collections. It is important that in any natural history collection, the DNA is preserved along with the physical phenotype. It is critical that collections from new environments (tropical, antarctic etc.) should be done in such a way that we collect DNA in a preserved form as well as samples of phenotype. We do not know what is going to happen in the next century. It would be such a tragedy if we were to walk into a museum in 50 years time and know that in the majority of samples the DNA was degraded.

What is the most useful form in which to store genotype? It would be ideal to preserve all specimens as viable germplasm, so that whole organisms could be obtained relatively easily. A limited amount of frozen material (whole organisms in the case of microfauna, eggs, ova and sperm) could then be used to produce large amounts of material from interbreeding of whole organisms. Although storage of sperm from domestic animals and seed storage from commercial cultivars has been well characterised, when it comes to sperm, eggs, whole organisms and embryos from a wide range of invertebrate species, there is such a variation of physiology from one species to another that considerable research is required to determine viable storage

conditions for each species. The difficulty and cost in collecting sperm or ova samples from many species also makes this impractical for comprehensive gene banks.

Tissue cultured cells stored in liquid nitrogen remain viable if regenerated every 5 to 10 years. Lymphoblastoid cell lines can be cultured to produce large amounts of human material, however suitable viruses need to be found to transform cells from other species. Cultured skin or muscle cells (fibroblasts) may be useful for other species, but obtaining suitable fresh skin samples is difficult. These may require considerable research to determine culture conditions for each new species. Storage of viable nuclei in any of the above forms should allow the regeneration of whole animals at some stage in the future.

The main drawback with storing tissues is that DNA is degraded by long term storage. Freezing should slow down this degradation considerably, but degradation is evident in DNA purified from mammalian blood samples that were stored at -60°C for 2 years. Accidental thawing will also result in degradation of DNA in these samples. There is not much known about long term storage of purified DNA. Once purified free of any detectable protein, any nuclease activity will be greatly reduced or non existent. Accidental thawing for short periods will not result in any significant degradation of DNA. This is the most preferable form for long term storage and has been adopted by the Gene Library (Mattick, Ablett & Edmonston, 1992). It may be preferable to keep DNA hydrated and we routinely store our samples in T.E. or 80% Ethanol. Purified DNA can be amplified by preparation of genomic libraries to provide unlimited amounts of material for future use. In this form it can be easily disseminated to other researchers. It may be possible to regenerate whole organisms from DNA or sequence data at some stage in the future.

USES OF PRESERVED GENETIC MATERIAL

As well as providing the raw material for molecular studies of biodiversity, gene banks facilitate this research in other ways. When raw materials are easily obtained from a central collection, new research is readily initiated, especially when the collection of samples in the field is time consuming, and requires some expertise. In vitro amplification allows the same sample to be used by countless workers, and eliminates the need for repeated sampling from wild species

(which could seriously threaten a restricted population). The availability of cloned genes will facilitate DNA sequencing studies and the development of DNA probes. These tools are revolutionising basic whole organism studies, as well as applied research. Species specific DNA probes are being used increasingly for species identification (Black et al., 1992). DNA probes directed against polymorphic sites in the genome provide new means for analysing the genetic diversity of populations in the wild. Such information is crucial to the development of rational conservation strategies. Hopefully, the use of DNA from the gene banks will generate many more such applications of DNA technology in wildlife research and management in the near future. DNA sequencing is now being used to explore invertebrate diversity, determine phylogenetic relationships and define speciation, as well as giving some insights into evolution (Crozier, 1992).

Many organisms have unusual features and biochemistries, which have developed to ensure survival in specialised environments. We are only just starting to utilise these results of millions of years of evolution. Wild species of the plant kingdom are providing information and genes to produce antibiotics, anti-cancer agents, diuretics, anti-parasite compounds, hormones, anaesthetics, cardiac and respiratory stimulants, and muscle relaxants. The exploration of the invertebrates for products useful to mankind is only just beginning (Beattie, 1994). With their enormous diversity of forms and function, the invertebrates promise to be an even more valuable resource than plants.

Although largely unaware of the scientific importance of the invertebrates, the general public is interested in new scientific developments that affect their everyday lives. Products of economic importance from invertebrates may be the key to improving the public image of the invertebrates. This in turn will lead to a higher profile for invertebrate research within the government and other bodies responsible for funding. There also needs to be a change in the public image of the invertebrate scientist from a figure sitting at a microscope counting hairs on a beetle's leg to someone out in the field, at the forefront of monitoring biodiversity and changes in the environment. Although traditional systematics is, and will, always be a major tool, its use in conjunction with the latest technologies, and production of invertebrate products useful to mankind

will facilitate an improvement in the public image of invertebrate scientists.

Technologies for the manufacture of biological products are becoming increasingly orientated to molecular systems. DNA from wild species, as the raw material for these industries, will become an increasingly important resource. Today, only a tiny percentage of the earth's organisms have been examined for their use in medicine and biotechnology. It is imperative to preserve DNA of unexplored species for future applications.

CONCLUSION

Biodiversity can be likened to a variation upon evolutionary themes. Each individual is unique and each species is unique, but there are relationships between. If one were to take a molecular biology perspective on biodiversity, it is going to be seen not as a series of absolutely unique genes or individuals, but as the individuals being unique because of the combination of their genes. As in music or art, a great symphony or a great painting is put together from a musical notes or colours which are just combinatorially rearranged. There is a whole spectrum of possibilities of colours and sounds but it is the combinations of them that make the painting or symphonies. This is what life is about. We need to preserve the molecular genetic information which tells us what combinations were made. Who knows what value that information will be in the future.

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